18.4. Monitoring IIMM patients using HLC assays

Immunoglobulin heavy/light chain (Hevylite, HLC) assays separately quantify the different light chain types of each immunoglobulin isotype (i.e. IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ, Chapter 9), and are assessed in pairs to produce HLC ratios (e.g. IgGκ/IgGλ) in the same manner as κ/λ sFLC ratios. IMWG consensus criteria for response and minimal residual disease recognise the value of HLC assays in a number of situations (Section 25.3.5) [1]. These include monitoring patients with oligosecretory disease or β-migrating monoclonal proteins, and providing evidence of tumour eradication in MM patients classified as MRD negative (by cell-based assays and imaging techniques). These, and other advantages are discussed below.

18.4.1. HLC assays are quantitative and non-subjective

HLC assays provide numerical results, are less labour-intensive and interpretation is less subjective compared with electrophoretic techniques. For many patients, accurate quantitation of monoclonal proteins by SPE is not possible for a variety of reasons (such as co-migration or dye saturation issues, Section 17.4). In such cases, HLC assays can provide clarity for monitoring patients. This is acknowledged in the 2016 IMWG consensus criteria for response and minimal residual disease assessment in multiple myeloma [1] which states that Hevylite can overcome the limitations associated with monitoring β-migrating monoclonal IgA by electrophoresis. One such patient is described in the clinical case history below.

For patients with a measurable monoclonal protein by SPE (M-spike), the relative change in the involved HLC or dHLC (involved - uninvolved) concentration has been shown to be equivalent to the relative change in the M-spike (Section 11.5.2) [3][4][5], Michallet et al. [5] compared responses assigned using Hevylite assays with those assigned using standard IMWG criteria for 463 newly diagnosed IgG and IgA IIMM patients enrolled in the IFM-2009 trial. Hevylite responses were based on changes in dHLC concentrations, and a complete response required normalisation of the HLC ratio (in addition to <5% bone marrow plasma cells). Post-consolidation therapy, there was a good agreement in assigned responses for ≤PR (79%) and ≥CR (92%) categories. By contrast, for patients with a very good PR (VGPR) by IMWG criteria, assigned HLC responses were concordant in only 45% (102/225) cases, with the remainder assigned as PR (18/225) or ≥CR (105/225) by HLC. The clinical outcome was significantly different between these HLC response categories (PR: PFS = 21.3 months; VGPR: PFS = 28.9 months; CR: median PFS not reached. p<0.0001), and the authors conclude that HLC responses correlate better with clinical outcome than current IMWG criteria.

Clinical case history 1

**Confirmation of disease progression in a patient with a broadly migrating IgA monoclonal protein.**
A 71-year-old female with a 9-year history of MGUS presented with anaemia and hypercalcaemia in January 2006. SPE showed a 48 g/L broadly migrating monoclonal protein in the γ-region. The monoclonal protein was identified as an IgAKκ intact immunoglobulin by sIFE (Figure 18.11). Total IgA levels were 47.2 g/L and there was an abnormal sFLC κ/λ ratio (7.0). A bone marrow biopsy indicated 40% monoclonal κ-restricted plasma cells. A bone survey revealed diffuse osteopenia, multiple small lytic lesions and a lesion consistent with plasmacytoma in one of the thoracic vertebrae. The patient was diagnosed with MM (Durie Salmon stage IIIA).

The patient was successfully treated and achieved a serum and urine IFE CR, which was sustained for 18 months. Subsequently, the patient relapsed with monoclonal IgAK (39.2 g/L), as detected by electrophoresis. She was successfully treated with Revlimid and dexamethasone, achieving a second CR (Figure 18.11, August 2010). Between August and October 2010, although electrophoresis remained negative, total IgA levels increased steadily and became abnormal. Concurrently, the patient developed a lung infection (Figure 18.11, October 2010). The elevation of serum IgA may have been attributable to the infection or, given the patient's history, due to disease relapse. However, IFE did not identify a monoclonal protein until January 2011.

Retrospective HLC analysis was performed on the equivocal samples taken between January 2008 and January 2011 (Figure 18.12). The IgAK/IgAλ HLC ratio decreased in response to treatment after the first relapse (January 2008) but did not normalise, possibly indicating residual disease. In the following months, HLC ratios increased steadily, supporting the existence of an abnormal clone despite the apparently normal IFE results.

The authors concluded that IgA HLC assays were particularly beneficial for monitoring this broadly migrating monoclonal protein, which was difficult to identify and quantify by serum electrophoretic techniques. In addition, IgA HLC was more sensitive than electrophoresis measurements for detecting disease relapse.

### 18.4.2. HLC assays to monitor oligosecretory patients

The 2016 IMWG consensus criteria for response and minimal residual disease assessment in multiple myeloma recognise that HLC assays can offer an additional means of monitoring oligosecretory patients (Section 25.3.5). This is based on a number of studies, which are discussed below.

In a study of 156 MM patients by Ludwig et al., an abnormal HLC ratio was identified at presentation in all 18 oligosecretory patients (7 IgG and 11 IgA). Similar findings were reported by Boyle et al. In a study by Young and colleagues IgG and IgA HLC analysis was performed on serial samples from 8 oligosecretory patients (5 IgA, 3 IgG) with sFLC <100 mg/L. In all 8 patients, the HLC ratio was abnormal at presentation and changes in the HLC ratio were in concordance with clinical assessment during follow-up. An example of an IgAK oligosecretory MM patient is shown in Figure 18.13. Although the patient initially responded to therapy (indicated by a fall in the IgAK/IgAλ HLC ratio), the HLC ratio remained abnormal. A subsequent increase in the HLC ratio suggested disease progression 445 days before a clinical relapse was confirmed.

### 18.4.3. HLC assays in MRD assessment

According to IMWG response criteria, the current definition of CR requires the absence of the monoclonal protein by IFE and <5% bone marrow plasma cells. However, this definition is not satisfactory for several reasons. Firstly, monoclonal
protein quantification by electrophoresis measures only the product of the secreting clone, and not all MM plasma cells are secretory [12]. Secondly, recycling of IgG by the FcRn receptor may prolong the persistence of IgG monoclonal proteins in the serum, causing IFE to remain positive long after the tumour has been eradicated (Section 3.5.3) [13]. Finally, IFE is a subjective technique, and interpretation can be difficult, leading to discordance in the response category assigned by different operators [14].

With the introduction of highly effective, novel therapies to treat MM, assays with increased sensitivity are required to improve the detection of residual disease and allow more precise comparisons of treatment responses [15][16]. With this in mind, IMWG response criteria have been revised to incorporate a number of new response criteria. Firstly, a more rigorous sCR category was defined, which incorporates sFLC analysis (Section 18.2.2) [10]. This was followed by new categories of minimal residual disease based on flow cytometry, gene sequencing or imaging techniques (Section 25.3.5) [11]. The guidelines also discuss a potential role for Hevylite to further improve the definition of MRD and recovery of normal plasma cell populations following therapy [8].

A number of studies have shown that abnormal HLC ratios may indicate residual disease in IMM patients whose electrophoresis results have normalised following therapy [6][8][17][18][19][20][23]. Ludwig et al. [8] used Hevylite to assess response in IgG and IgA MM patients. A total of 100 IgG and 56 IgA patients were followed for a median of 46.1 months. Hevylite results were compared with data from SPE, IFE, nephelometry and sFLC assays. Following induction therapy, a CR or sCR was observed in 31 patients. Residual disease was indicated by an abnormal HLC ratio in 8/31 patients – in four of these HLC was the only abnormality, and in the remaining four, abnormal HLC and sFLC ratios were observed. Figure 18.14A shows an example of an IgAκ MM patient who achieved a CR during follow-up [8]. The monoclonal IgAκ became undetectable by IFE, but the IgAκ/IgAλ HLC ratio remained abnormal due to HLC pair suppression. After further follow-up, the HLC ratio normalised. In this case, the κ/λ sFLC ratio showed a similar trend to the HLC ratio throughout the study.

Miyazaki and colleagues [22] studied HLC results in 45 MM patients who had responded to treatment and achieved negative sIFE and normal sFLCs. In this group, 7 and 9 patients had abnormal HLC ratios and HLC pair suppression, respectively. The authors concluded that the addition of Hevylite measurements to IFE and sFLCs improve the sensitivity of residual disease detection.

It has been reported that patients with an abnormal HLC ratio at maximum response have a significantly worse outcome than patients in whom the HLC ratio normalises [8][23] (Chapter 20). An abnormal HLC ratio may be the result of HLC pair suppression (as illustrated in the case by Ludwig et al. discussed above). HLC pair suppression is an important prognostic marker, and has been shown to identify patients with inferior progression-free survival [23] and overall survival [24] (Chapter 20).

HLC analysis may have an important role in the definition of a MRD-negative state: in particular, an increase in the uninvolved HLC concentration to normal levels following successful therapy may indicate immune recovery. This was mentioned in recent IMWG guidelines [1], which discuss how normalisation of HLC ratios in combination with the absence of MRD (by cell-based assays and imaging techniques) may represent a composite endpoint. Such an endpoint may confirm the eradication of tumour cells from all compartments and the recovery of normal plasma cell populations. An initial prospective study by Campbell et al. [25] concluded that normalisation of both HLC and FLC ratios serves as a useful surrogate marker for MRD-negativity by flow cytometry, and correlates with improved MM progression free survival (Section 20.6). Further study of Hevylite as a marker of MRD is encouraged.

### 18.4.4. HLC assays improve detection of relapse

Ludwig et al. [8] reported three MM patients in whom a HLC abnormality pointed to imminent relapse while IFE was still negative. Data from one of the three patients is shown in Figure 18.14. Following induction therapy, the patient with IgAκ MM initially achieved a CR and the IgAκ/IgAλ HLC ratio was normal [8]. Seven months later, the HLC ratio became abnormal due to pair-suppression of IgAλ (IgAκ was still within the normal range). Subsequently, IgAκ became elevated, with further reductions in the uninvolved HLC pair, confirming disease evolution. IFE became positive 5.5 months after the HLC ratio became abnormal. It is noteworthy that, although the κ/λ sFLC ratio was abnormal in this patient at diagnosis, it normalised following treatment.
and remained normal at relapse. This highlights the importance of measuring both FLCs and intact immunoglobulins during follow-up.

Chae et al. also reported two MM patients that were considered to have complete response (with a normal SPE, sIFE and \(\kappa/\lambda\) sFLC ratio), but an abnormal HLC ratio indicated relapse 1.5 and 3 months earlier than sIFE became positive. As such, the HLC ratio was an early indicator of relapse.

Other examples where abnormal HLC ratios or increasing dHLC values have provided an early indication of relapse have been published elsewhere. Bradwell et al. reported on 5 IgG MM patients who achieved a CR by IFE with normalisation of the IgGk/IgGA HLC ratio. In 3 of 5 patients, subsequent relapse was indicated earlier by abnormal HLC ratios. In an IgA patient, abnormal HLC ratios indicated a slow relapse more than a year before IFE became positive.

18.4.5. Discrepancies between HLC and IFE during follow-up

Ludwig et al. observed a normal HLC ratio in 12 of 35 MM patients who achieved a near complete response (nCR: 100% monoclonal protein reduction by electrophoresis, but IFE-positive), or a very good partial response (VGPR: at least 90% serum and urine monoclonal protein reduction) following treatment. This discrepancy between HLC analysis and IFE may be due to the recovery of polyclonal immunoglobulins producing a normal HLC ratio, despite the persistence of small amounts of monoclonal immunoglobulin. While continuing low levels of a monoclonal protein may reflect residual disease, it has been shown that a normal HLC ratio predicts a good patient outcome, irrespective of IFE positivity (Chapter 20).

In IgG patients specifically, persistence of small amounts of monoclonal IgG is partly the result of a prolonged half-life due to recycling by FcRn receptors, causing IFE to remain positive after the tumour has been eradicated. This is an issue previously reported by Waldmann et al. (Chapter 3) and demonstrated in Figure 18.15. Consistent with this, discrepancies between IFE and bone marrow immunophenotypic responses can be observed during follow-up.

HLC ratios are not affected by the variable metabolism of IgG and for the detection of residual disease, they demonstrate a better agreement than sIFE with flow cytometry results (Figure 18.16). Others have similarly found a good correlation between HLC and flow cytometry during serial monitoring including those patients treated with novel agents.
18.3. Other uses of sFLC analysis in IIMM response assessment

Figures

Figure 18.11. SPE, IFE and HLC analysis during the disease course.

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Figure 18.12. HLC and FLC results following the first relapse of disease.

Dashed lines indicate the upper limits of the reference ranges for total IgA (black), IgAκ/IgAλ HLC ratio (red) and κ/λ sFLC ratio (blue). (Graph generated using published data).

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- 18.4.1. HLC assays are quantitative and non-subjective

Figure 18.13. An increase in the HLC ratio indicates disease progression 445 days before relapse is clinically confirmed in a patient with oligosecretory IgAκ MM.
The upper limit for the IgA HLC ratio reference range is indicated by the dashed line. MR: maximum response, PD: progressive disease [7].

**Figure 18.14. Utility of IgA HLC for monitoring IgA MM.**

(A) IFE became negative when an abnormal IgAκ/IgAλ HLC ratio indicated residual disease. The HLC ratio became normal with further follow up. (B) IFE and the IgAκ/IgAλ HLC ratio became normal at the same time, but on subsequent follow up, IgA HLC ratio became abnormal, indicating relapse when IFE was still normal. (Reprinted by permission from Macmillan Publishers Ltd: Leukemia [8], copyright 2013).

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- 18.4.2. HLC assays to monitor oligosecretory patients
- 18.4.3. HLC assays in MRD assessment
- 18.4.4. HLC assays improve detection of relapse

**Figure 18.15. Theoretical patient with persistence of low-level monoclonal IgG due to FcRn receptor recycling.**
Although monoclonal IgGκ production by the tumour ceases following treatment, low levels of IgGκ are still detected by CZE/IFE due to prolonged IgG half-life.

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- 18.4.5. Discrepancies between HLC and IFE during follow-up

**Figure 18.16.** Comparison between flow cytometry (FC), serum immunofixation (sIFE), HLC ratios (Hevylite) and κ/λ sFLC ratios (Freelite) for assessment of minimal residual disease (MRD) in IMM patients.

Results analysed at 3 time-points (pre-ASCT, MRD1; post-ASCT, MRD2; and post-consolidation, MRD3) showed a good correlation between HLC ratios and FC. At each time-point, sIFE assigned a greater percentage of MRD-positive patients than HLC or FC. [33] (Obtained from Haematologica Journal website: haematologica.org).

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- 18.4.5. Discrepancies between HLC and IFE during follow-up

**References**


